What do we know about actinides-protein interactions?
G Creff, C Zurita, A Jeanson, G Carle, C Vidaud, C den Auwer

To cite this version:
G Creff, C Zurita, A Jeanson, G Carle, C Vidaud, et al.. What do we know about actinides-protein interactions?. Radiochimica Acta, 2019, 10.1515/ract-2019-3120. hal-03592400

HAL Id: hal-03592400
https://hal.science/hal-03592400
Submitted on 1 Mar 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
What do we know about actinides-protein interactions?

G. Creff\textsuperscript{a}, C. Zurita\textsuperscript{a}, A. Jeanson\textsuperscript{a}, G. Carle\textsuperscript{b}, C. Vidaud\textsuperscript{c}, C. Den Auwer\textsuperscript{a}

\textsuperscript{a}Université Côte d’Azur, CNRS, UMR 7272, Institut de Chimie de Nice, 06108 Nice, France
\textsuperscript{b}Université Côte d’Azur, CEA, UMR E-4320 TIRO-MATOs, 06100 Nice, France
\textsuperscript{c}CEA DRF, Marcoule, 30207 Bagnols sur Cèze, France

Abstract. Since the early 40s when the first research related to the development of the atomic bomb began for the Manhattan Project, actinides (An) and their association with the use of nuclear energy for civil applications, such as in the generation of electricity, have been a constant source of interest and fear. In 1962, the first Society of Toxicology (SOT), led by H. Hodge, was established at the University of Rochester (USA). It was commissioned as part of the Manhattan Project to assess the impact of nuclear weapons production on workers' health. As a result of this initiative, the retention and excretion rates of radioactive heavy metals, their physiological impact in the event of acute exposure and their main biological targets were assessed. In this context, the scientific community began to focus on the role of proteins in the transportation and \textit{in vivo} accumulation of An. The first studies focused on the identification of these proteins. Thereafter, the continuous development of physico-chemical characterization techniques made it possible to go further and specify the modes of interaction with proteins from both a thermodynamic and structural point of view, as well as from a their biological activity. This article reviews the work performed in this area since the Manhattan Project. It is divided into three parts: first, the identification of the most affine proteins; second, the study of the affinity and structure of protein-An complexes; and third, the impact of actinide ligation on protein conformation and function.

Key words: Actinides, Osteopontin, Calmodulin, Transferrin, Ferritin, Albumin, Fetuin, Phosvitin, Metallothionein, Hemoglobin, Centrin, spectroscopies, FTIR, EXAFS, TRLFS
Introduction

Some metal ions, constituting what is called the "metallome", are essential and ubiquitous in biological systems. The occurrence of metal ion complexes in biology is further increased by the diversity of potential biological ligands contained in the cell machinery. Some metal elements are essential for life, others are or may be essential at trace levels, and others are non-essential. But in any case, toxicity depends on their concentration. As a general rule, most essential metals are located in the upper part of the periodic table (mainly the first row), although there are some exceptions. Actinide elements (An) are located at the very bottom of the periodic table and are non-essential elements for life. The actinide family is composed of 15 metallic elements, corresponding to the filling of the 5f layer (from actinium, n°89, to lawrencium, n°103). All actinide isotopes are radioactive, have a high tendency for hydrolysis and present a wide range of redox states (+III, IV, V and VI for the most commons). These radioelements are hard acids, according to Pearson classification, having a particular affinity for hard bases: hydroxide, carbonates, carboxylates, phosphates etc. Uranium (U) and thorium (Th) occupy a specific position within this family because they are the only elements at the origin of the three natural decay chains present today in the earth crust. They were discovered in 1789 by the German chemist Martin Heinrich Klaproth for U and in 1828 by the Swedish chemist Jöns Jakob Berzelius for Th. However, the toxicity of these elements was considered much later, a few decades after the discovery of radioactivity in 1896 by Henry Becquerel and shortly after the discovery of the first transuranic elements from anthropogenic origin, which are neptunium (Np), in 1940, by Edwin Mac Millan and plutonium (Pu), in 1941, by Glenn T. Seaborg (both from Berkeley, CA, USA). Thus, actinide toxicity research, including biokinetics and biochemistry, started in 1942 together with the expansion of the nuclear industry during the second half of the Second World War.

In the framework of the Manhattan Project (1942 – 1946), investigation of the health effects associated with radiochemistry and weapons production was initiated. In 1943, the same year
as the construction of the full-scale plutonium production reactor (Hanford B reactor, WA, USA), the University of Rochester was commissioned by the Atomic Energy Commission to assess the potentially deleterious effects of atomic energy production on human health. Many researchers, from various scientific disciplines, worked on the toxicity of U as well as fluorides, mercury and other chemicals used in the production of nuclear weapons. In 1962, Professor Harold C. Hodge created the first Society of Toxicology (SOT). ([1] Morrow, 2000)

Early studies described the retention as well as the excretion rates of actinides depending on the element itself and its redox state, but also as a function of its mode of administration (injection, ingestion or inhalation). ([2] Durbin, 2010) The main target organs of these elements were identified and the toxic impact according to exposure or injection dose was assessed. The sensitivity of various model living organisms was also explored: mice, rats, rabbits, dogs and monkeys (juveniles and mature, males and females). Based on those works, international committees such as the International Commission on Radiological Protection (ICRP) have defined exposure limits for nuclear workers and for the public. (ICRP reports, 1959 [3], 1979 [4], 1980 [5], 1986 [6], 1993 [7], 1995 [8])

It is interesting to note that lanthanide elements (Ln) corresponding to the filling of the electronic layer 4f (therefore 1 raw before the actinides in the periodic table), have often been used as non-radioactive (thus, more easily manipulated) “analogues” of the actinides. ([9] Choppin, 1983; [10] Vidaud, 2012) Although they may present some chemical similitudes with their unstable 5f neighbors, their chemistry is mostly limited to oxidation state +III and therefore often differs from that of the actinides. This is particularly true for early actinides for which the most stable oxidation states span from +IV to +VI. This considerably limits the validity of the analogy to oxidation state +III. Nonetheless, lanthanides have often been considered as actinide(III) analogues, “in a first approximation”, because they are all considered as hard acids and large cations with high coordination numbers. For both families, they all have a strong tendency for hydrolysis at physiological pH, although this is modulated by oxidation state.
Uranium is the most widely studied actinide to date, certainly because of its natural abundance in the earth’s crust and hydrosphere, but also because of its privileged position in the nuclear cycle of energy production. In biological redox media (O₂, H₂O), independent from its physico-chemical form and from its mode of internalization (solid via the epidermis, particles via inhalation or dissolved in drinking water, etc.) uranium is easily dissolved, oxidized and then predominantly exists under its main redox state +VI, corresponding to the oxocationic form UO₂²⁺ (uranyl). Unlike other actinides, U is the most easily excreted element (about 70%, 6 days after injection). ([11] Hamilton, 1947; [12] Durbin, 1957) The 30% fraction retained in the body is shared between the bone (10-15%) and the kidneys (12-25%). ([13] Hamilton, 1948) For all other actinides, regardless of their redox state, the liver and skeleton are mainly targeted. Np(V) (in its oxocationic form NpO₂⁺, neptunyl) is mainly present in bone (between 30-50%). It is also present in the liver, but to a lesser extent (2-10%). Its excretion rate is quite high, around 40%, after one day. ([14] Durbin, 1987) An(III) (americium, Am and curium, Cm) are retained between 30 and 50% in the skeleton, and up to 50% in the liver. Their excretion rate is very low, being less than 1%, one day after injection. ([15] Ansoborlo, 2006; IRCP reports 1993 [7], 1995 [8], 1998 [16]) An(IV) (mainly Th, Pu and Np) have the highest degree of affinity for the skeleton with retention rates up to 70%, 50% and 30-50%, respectively and low urinary excretion rates (as An(III)), from 0.4% for Pu(IV) to 6% for Th(IV) after 5 days. ([15] Ansoborlo, 2006) From this short summary, biokinetic data appear to be highly dependent on the element itself and its oxidation state. Another important parameter to assess toxicity is isotopy because actinides have a double toxicity: a radiological toxicity that dominates for short half-lives (depending also on the decay mode) and chemical toxicity that becomes predominant for the largest half-lives (as for ²³⁸U for instance, 4.5 billion years). During the years following the Manhattan Project (1942-1946), biokinetic and toxicological data were considered sufficient for radiation protection purposes, and less attention was given to the understanding of actinides biochemistry in blood, tissue fluids, and more generally in the mammalian body. During that time, mechanistic studies have been rather scarce. Knowing
that the chemical reactions of actinide ions in warm (37°C), nearly neutral (pH 7.4), dilute saline medium of the mammalian body are dominated by hydrolysis and complexation by metabolites, vectorization and accumulation mechanisms are very difficult to describe.

Proteins, large assemblies of amino acids chains, are ubiquitous macromolecules present in any eukaryotic cell and whose functions are extremely diversified. Among them, some could be found in both animal and plants cells (like calmodulin, ferritin, albumin and centrin), while others are specific to the animal cells (like some hyperphosphorylated proteins such as phosvitin or osteopontin). Metalloproteins (which represent more than one third of all proteins) have attracted the attention of chemists and biochemists, particularly since the 1950s when John Kendrew and his collaborators elucidated the first X-ray crystal 3D-structure of a protein, sperm whale myoglobin, indicating the presence of an iron atom. ([17] Kendrew, 1958) Since (metallo)proteins are involved in the various biological processes of any living system (as enzymatic processes, transportation, electron transfer, oxygen fixation and transportation, transcriptional activation, chelation or metal regulation...) and hold a key position in metabolism, they occupy a privileged position in the investigation of the mechanisms of actinide toxicology. Among the possible metal co-factors, iron, copper and manganese, with varying redox states, zinc, calcium and magnesium, at oxidation state +II, are the most common metal cofactors. To a lesser extent, nickel, cobalt, molybdenum, vanadium or tungsten are also found in the active site of the proteins. These metal co-factors can have a structural or a catalytic role. ([18] Degtyarenko, 2005) Amino acids are the basic building blocks of proteins controlling primary, secondary, tertiary and even quaternary structures of the protein that are the keys of their activity. The imidazole ring of histidines, the thiolate group of cysteines, the carboxylate group of aspartates and glutamates and the phenolate function of tyrosines are the main groups involved in the chelation of metallic ionic species. However, in view of the diversity of metalloproteins, most amino acid side chains and the carbon skeleton (with oxygen from amide groups or deprotonated amines) can be involved in the chelation of
metal ions. In addition to these donor groups present on the protein itself, many organic cofactors can have a coordinating or chelating function (as porphyrin). Water and inorganic carbonate anions may also be present as ligands of metal ions.

As for many heavy metals (a loosely defined group of elements that include transition metals and some metalloids like arsenic, cadmium, mercury, lead and chromium) ([19] Appenroth, 2010), the molecular mechanisms underlying the chemical toxicity of actinides are poorly understood. It is admitted that heavy atoms interfere with the physiological activity of specific, particularly susceptible proteins, either by displacing essential metal ions or by forming a complex with different functional side chain groups of the metalloprotein. ([20] Lemire, 2013)

Recent studies have revealed an additional mode of metal action targeted on unfolded proteins. Particular heavy metals and metalloids have been found to inhibit the in vitro refolding of chemically denatured proteins, to interfere with protein folding in vivo and to cause aggregation of nascent proteins in living cells. ([21] Tamas, 2014) By interfering with the folding of the nascent or non-native (unfolded) proteins, heavy metals in general may profoundly affect protein homeostasis and cell viability.

This report reviews current knowledge on actinide-protein interaction. In the first section, the identification of actinide’s target proteins, mostly in serum, but also in target organs will be presented. In the second part of the paper, the thermodynamic and structural determination of the actinide’s complexation sites will be summarized. Finally a third section, smaller, deals with recent works on the impact of actinides on protein conformation and function.

I. Identification of actinide’s target proteins

Among the potential actinide target proteins, the proteins involved in metals transport (in serum) and homeostasis and the proteins present in the main target organs (liver, kidneys and skeleton) have attracted a great deal of attention from the scientific community. We can see
that the vast majority of studies devoted to the identification of target proteins are limited to the major actinides U(VI) and Pu(IV), although some studies have also focused on An(III).

- Serum proteins:

Serum proteins are of particular interest since they play an essential role in the transportation of metals and thus actinides, to their different target organs. These proteins have therefore attracted most of the interest from the scientific community. They have been classified into two groups, according to electrophoresis experiments developed by Arne Tiselius ([22] Tiselius, 1937), Nobel laureate in 1948: albumin (60%) and globulins (α1, α2 including fetuin A, β including transferrin, γ or immunoglobulins, hiding more than 10 000 proteins). Electrophoresis is a technique that remains very commonly used in medical diagnosis.

The first published data on the interaction of actinides with serum proteins dates back to the 40s. After the Manhattan Project, Muntz and Belialev ([23] Muntz, 1947; [24] Beliayev, 1959) showed, through electrophoresis, that Pu(VI) is mainly associated with proteins from the β-globulin group. In their study published in 1965 in Nature, Boocock and his collaborators also identified transferrin (Tf) as the main target of Pu(IV) (the main redox state of Pu in vivo) in serum in vivo after 0.5 hours of exposure to this actinide. ([25] Boocock, 1965) This single-chain glycoprotein has a molecular weight around 79 kDa and consists of 679 amino acids. In “normal” functioning, Tf can complex up to two Fe(III) cations in its two different lobes, namely C and N, leading to the lobes’ closure. This conformation change ensures its recognition by the Tf membrane receptor Tf-R and then its cellular internalization (schematized in Figure 1). Turner et al. confirmed this preliminary result in 1968, with an in vitro study of the complexation of Pu(IV) in equine serum. ([26] Turner, 1968)

Figure 1. TfR-mediated intracellular iron uptake. (a) Once bound to two Fe(III) ions, the complex Fe₂Tf is recognized by the TfR membrane receptor and internalized via endocytosis; metal release occurs at
endosomal pH. This is the proposed pathway for intracellular uptake of exogenous metals such as Cm(III). (b) Metal binding site of the Tf N-Lobe, with Tyr 188, Tyr 95, His 249, Asp 63 and a synergistic carbonate anion participating in the coordination of Fe(III).14 (c) Structure of the (Tf)−TfR complex, with TfR in green, Tf N-lobes in red and Tf C-lobes in blue. ([90] Sturzbecher-Hoehne, 2013)

They showed not only that Tf is the main target protein in serum, but also highlighted the role of bicarbonate as synergistic anions in the complexation of Pu(IV) by the protein. Later, Taylor and colleagues ([27] Taylor, 1987) suggested that Pa(V), Np(V) and Th(IV) are also mainly transported by Tf in blood. The authors suggested the internalization of these actinides into the liver cells and also their complexation with ferritin (F), another cellular protein involved in Fe(III) storage in mitochondria. They argued that the high affinity of Pa(V) and Np(V) for Tf can be explained by a reduction into Pa(IV) and Np(IV) in physiological conditions. ([28] Taylor, 1998) This hypothesis is thermodynamically sounded since those redox potentials fall within the range of redox potentials expected in aqueous media, respectively -0.1V for Pa(V)/Pa(IV) ([29] Fried, 1954) and +0.596V for Np(V)/Np(IV). ([30] Kihara, 1999) Wirth came to the same conclusion after having demonstrated a predominant complexation of Np(V) by Tf. ([31] Wirth, 1985)

The affinity of An(III) such as Ac, Am, Cm and californium (Cf) for Tf has been less clearly established ([32] Duffield, 1986), but only 30% of Am(III) and Cm(III) appears to be associated with Tf. ([33] Cooper, 1981)

The case of U(VI) is more ambiguous. In blood, it forms various complexes, mainly with carbonate ions (60%) and proteins (30%). Among the main serum proteins presenting an affinity for U(VI), one finds fetuin A (FETUA) and albumin (HSA) although this latter has a lower affinity than Tf. In the case of HSA, its huge concentration in serum (about 40 g/L) explains its binding capacity for U(VI), simply by displacing the thermodynamic equilibrium in favor of complexation. FETUA protein has a relatively low concentration in serum (40 times lower than HSA) but an apparent affinity constant \( K_D \) (\( K_D = [\text{Ligand}_{\text{free}}] \) when \( [\text{UO}_2^{2+}] = [\text{UO}_2^{2+} \cdot \text{Ligand}] \))
meaning that $K_D$ equals [Ligand\textsubscript{free}] leading 50% of [UO\textsubscript{2}^{2+}] occupancy, \cite{Abergel2017} for U(VI) around 30 nM, far higher than the Tf affinity constant equal to 2.80 µM. \cite{Basset2013} Among a cocktail composed of 15 proteins selected both for their relative abundance in blood and for their potential affinity to U(VI), a calculation shows that FETUA could bind 80% of the U(VI) present in the serum, while HSA, Tf, $\alpha_2$ macroglobulin and haptoglobin bind only 15% of the remaining oxocation. These results complete previous work where the U(VI) target serum proteins were screened by coupling two-dimensional chromatography of serum proteins with Time Resolved Fluorescence Spectroscopy (TRFS) of U(VI)-complexes and proteomic analysis. \cite{Vidaud2005} Ten major target proteins were primarily identified, FETUA was identified later. This protein is secreted into the blood at around 1 mg/mL by liver cells. It is known to bind strongly to "young" apatite, to inhibit apatite precipitation and to transport calcium phosphate particles to the bone surface, thus contributing to bone formation and accretion, and constituting 25% of the fraction of non-collagenous proteins in the bone matrix. It is not excluded that FETUA acts, not only as the major carrier of U(VI) in serum, but also as an agent promoting the biomineralization of U(VI) in bone. \cite{Bourgeois2015} More recently, isoelectric focusing coupled with inductively coupled plasma mass spectrometry (CE-ICP-MS) has been successfully used to characterize the interaction of U(VI) with FETUA, Tf and Alb along competitions between these proteins, confirming FETUA as the preferred U(VI)-target in serum. \cite{Huynh2015}

\textit{In silico} approaches were also used to identify U(VI) protein targets, in particular by gathering structural information of the uranyl-first shell atoms in proteins from two structural data bases (the Protein Data Bank (PDB) and the Cambridge Structural Databank (CSD)). \cite{Pible2006} This approach allowed to screen the potential U(VI)-binding sites in proteins of known crystallographic structures. For instance for the C-reactive protein (serum protein appearing during acute inflammation in the body), this screening methodology highlighted the fact that the U(VI)-binding site is very similar to the Ca(II)-binding site, with a 100 times greater affinity.
for U(VI) than for Ca(II). ([40] Pible, 2010) In 2014, Zhou et al., based on theoretical screening of U(VI) affine proteins (as illustrated in Figure 2), proposed the use of a U(VI) super-affine mutant protein (SUP) with an affinity constant of about 7.4 fM to recover U(VI) from seawater. ([41] Zhou, 2014)

Figure 2. The main steps in computational screening and design of uranyl-binding proteins.

Primary steps in the computational screening and subsequent design of super-uranyl-binding proteins. The desired coordination features are identified (step 1), the protein database is surveyed and a protein selected for further study (step 2), possible mutations for the protein are considered (step 3), potential uranyl binding sites are sought (step 4), and uranyl coordination geometries for the selective potential binding sites are evaluated and scored (step 5). If no hits are identified, the computational algorithm returns to step 2. ([41] Zhou, 2014)

- Proteins involved in bone remodeling:

After in vivo contamination, uranium has been found to be mostly located in calcifying zones of bones in exposed rats. It accumulates in the endosteal and periosteal area, in calcifying cartilage and in recently formed bone tissue along trabecular bone. ([37] Bourgeois, 2015) Milgram and its co-authors showed that osteocalcin protein (synthesized by the osteoblast cells and involved in calcium binding) and sialoprotein, two non-collagenous proteins involved in bone remodeling, are inhibited following exposure to U(VI). ([42] Milgram, 2008) Also, the excretion level of osteopontin (OPN), a phosphorylated protein secreted by bone cells and involved in mineralization processes, decreases dramatically in the case of uranium exposure. ([43] Prat, 2011) This protein, together with FETUA have been identified as bone protein actinide targets. ([44] Safi, 2013; [45] Qi, 2014; [35] Basset, 2013) These results were later confirmed by using capillary electrophoresis techniques coupled to inductively-coupled mass spectrometry. ([38] Huynh, 2015; [46] Huynh, 2016)
Kidney proteins:

Kidneys are the main target organ of uranium which is a well-known nephrotoxic agent. The renal function is altered by the uptake of small uranyl particles that are filtered through renal glomeruli and present at the level of the proximal tubule epithelium. The carbonate, citrate and also phosphate complexes of uranyl were suspected to be responsible for the observed cytotoxic effects. ([47] Chevari, 1968; [48] Cooper, 1982; [49] Carrière, 2004) Transcriptomics and proteomics are two powerful approaches for identifying the molecular events (modulation of gene and protein expressions respectively) involved in the cellular response to U(VI) exposure. Such transcriptomic studies on human kidney cells have shown that the expression of several genes were up- or down-regulated after U(VI) exposure which is also dependent on its concentration. Combined with transcriptomic analyses, it has led to the identification of the U(VI) modulated proteins. ([50] Taulan, 2006; [51] Prat, 2006) But having a modulated expression does not imply that those proteins are U(VI) targets. To this purpose, the implementation of an original chromatography setup using the immobilization of U(VI) by aminophosphonate groups grafted on a chromatographic phase led to the capture of U(VI) affine proteins. ([52] Basset, 2008) Using this technique, Dedieu et al. identified 64 U(VI)-affine proteins in soluble extracts of human kidney cells (HK-2). ([53] Dedieu, 2009) Also in 2009, Frelon et al. explored rat kidney proteins-U(VI) interactions using isoelectric focusing gel electrophoresis and by laser ablation ICPMS. ([54] Frelon, 2009) They evidenced that U(VI) can bind few proteins and that most of the uranium-protein complexes in this organ are non-covalent but electrostatic ones.

Liver proteins:

Because the liver is not the main target organ of U(VI), data on specific U(VI) affine proteins in this tissue are rare. Surprisingly, Pu(IV) and Am(III) affine liver proteins have not been described in detail, although the liver represents one of the main target organs for An(III) and
An(IV). ([55] Taylor, 1989) However, similar to the aforementioned chromatographic methodology, Aryal et al. used the immobilization of Pu(IV) on nitrilotriacetate (NTA) groups to capture Pu(IV)-affine proteins from PC12 cell lines from the rat adrenal gland, another target organ of plutonium. The authors identified 7 potential Pu(IV)-binding proteins ([56] Aryal, 2011): the 78 kDa glucose-regulated protein precursor, the nucleoside diphosphate kinase, the B. actin, the gamma 1 propeptide-like, the galectin-1, a pyruvate kinase and the complement component 1 Q, most of which are calcium binding proteins.

- Other target organs:

Proteomic approaches on the tumor cell line PC12 from the adrenal gland of rats have led to the identification of affine proteins of the Pu(IV) cation in this organ. ([58] Aryal, 2012) The proteins involved in the transportation of Ca(II), and more generally of divalent transition metals which also have anti-apoptosis functions, have a proven affinity for Pu(IV). This suggests that Pu(IV) could selectively stimulate these proteins and promote the development of cancers. Calmodulin (CaM) is a good candidate among the target proteins of Pu involved in Ca(II) binding. In a study published in 1997, Seeger et al. used small angle neutron scattering (SANS) to show that the complexation site of Pu(III) corresponds to that of Ca(II) within the protein. ([59] Seeger, 1997) Calmodulins with a specifically tuned affinity for U(VI) have also been recently successfully engineered in order to recover U(VI) from seawater which represents an important (in volume) uranium reserve. ([60] Le Clainche, 2006, [61] Pardoux, 2012)

Quite recently, some authors have started to take interest in phosphorylated protein binding sites ([44] Safi, 2013, [45] Qi, 2014, [62] Creff, 2015) which, surprisingly, were almost absent from the actinide-protein literature. In a work on the identification of U(VI) target proteins in the ovaries of zebrafish, Eb-Levadoux highlighted the importance of phosphorylations in the ability of proteins to bind U(VI). ([63] Eb-Levadoux, 2017) He showed that the 20 potential ovary target proteins of U(VI) are also proteins involved in Fe(III) homeostasis and that they are
involved in three main biological processes: regulation of oxidative stress, cytoskeleton structure and primary embryonic development.

Finally U(VI), in the form of U(VI) acetate, also appears to affect DNA-binding proteins such as "zinc finger" proteins, with an inhibition of the protein function in the presence of the exogenous metal, most certainly via a direct but non-specific binding of the metal to the protein. ([57] Hartsock, 2007)

- Other proteins non-involved in target organs metabolism:

If the very large majority of research works published up to now concerns the interaction of actinides with proteins involved in the transport via the serum and in the metabolisms in the main target organs (skeleton, kidney and liver), few studies have focused on proteins involved in trans-membranous transport and in the penetration pathways of these toxic metals. For example, Barkleit was interested in the proteins involved in digestion and more specifically in alpha amylase, an enzyme found in saliva. Using spectroscopic and thermodynamic modelling tools, he explored the interaction of this protein with uranium (VI) ([64] Barkleit, 2018) as well as with curium (III) and its non-radioactive surrogate. (Eu (III)) ([65] Barkleit, 2016, [66] Wilke, 2017, [67] Barkleit, 2017) On the other hand, Malard and his collaborators identified by proteomic analysis the response of human lung cells exposed to uranium. ([68] Malard, 2005)

Among 81 proteins they were able to show that cytokeratins CK8 and CK18 are involved in the toxicity mechanisms associated with uranium exposition in lung cells. Still in the family of proteins involved in the mechanisms of respiration, Wan identified the impact of uranium on the structure and function of cytochrome b5, a heme protein capable to bind cell membranes and involved in electron exchanges. ([69] Wan, 2012a, [70] Wan, 2012b) Finally the role of glycophorin A (GpA), a trans-membranous protein, was highlighted in the aggregation and hemolysis phenomena observed in human erythrocytes exposed to natural thorium. ([71] Kumar, 2010)
II. Characterization of complexation sites using modelling, thermodynamics and spectroscopy

The identification of actinide major target proteins opened the door to a new field of research focusing on thermodynamic and structural characterization of the binding sites of these macromolecules. This is essential for defining and understanding the impact of An on protein structure and function. Overall, this is the entryway into the understanding of An transport and accumulation mechanisms in the cells and tissues. While the development of spectroscopic techniques has contributed significantly to the latest advances in this field, the results remain incomplete. For thermodynamic data in particular, values are condition dependent and often not even comparable. The systematic determination of comparable affinity constants remains difficult until now. In addition, the choice of the model system or medium is essential and strongly influences data determination. Composition, pH and concentration are all factors that influence metal speciation and will induce deviations from in vivo conditions. Nevertheless, trends in An affinity have been drawn and chelating groups, or families most often involved in protein complexing sites, have been identified.

Most of the proteins involved in the complexation of U(VI) have structural similarities. They contain carboxylic acids of the aspartate or glutamate amino acids. ([72] Van Horn, 2006) Amide groups also seem to contribute significantly to the first coordination sphere of the metal. Very recently, Carugo et al. pointed out that U(VI) tends to interact with carboxylic acids from aspartic and glutamic residues, most often via monodentate bonding. ([73] Carugo, 2018) Water molecules may also complete the metal's coordinating sphere.

- Transferrin (Tf):

In the case of Tf, by far the most studied protein for its role in actinide transfer and all-body diffusion, the combination of various spectroscopic tools has been implemented. For this
protein, Fe(III) binding sites were already well-defined and reported in the Protein Data Bank. ([74] Princiotto, 1975) According to Yang’s work, two Fe(III) cations are bound to apotransferrin via two tyrosine residues (Tyr95 and Tyr188) in the N2-subdomain (a bidentate sulfate and a bidentate carbonate, as synergistic ion, complete the coordination sphere) and four residues two tyrosine, one aspartate and one histidine (Tyr426, Tyr517, Asp392 and His585) (a bidentate carbonate complete the coordination sphere), as schematized in Figure 3. ([75] Yang, 2012)

Figure 3: (a) Representation of the Fe$_3$Fe$_C$-hTF structure with subdomains N1 in blue, N2 in green, C$_1$ in yellow, C$_2$ in red, and peptide linker in purple. Fe(III) ions are represented as sphere models in brown. Two N-acetylglucosamine moieties (NAG and NAG') are represented as sphere models, (b) Coordination of the Fe(III) in the N-lobe of FeNFeC-hTF. (c) Iron binding center in the C-lobe of FeNFeC-hTF. ([75] Yang, 2012)

Boocock and Turner investigated the similarity between Fe(III) and Pu(IV) coordination by observing the displacement of Pu(IV) outside the Tf binding site, in the presence of an excess of Fe(III). ([25] Boocock, 1965; [26] Turner, 1968) On the other hand, in 1968 Stevens et al. underlined the importance of the conformation of the protein complexation site for its ability to bind Pu(IV). ([76] Stevens, 1968) By eliminating the glycopeptide chain from Tf, they highlighted the fact that Pu(IV) is not able to bind the protein while Fe(III) does not seem to be affected by this modification. Duffield and his co-authors also showed in a preliminary approach which used UV-visible absorption spectroscopy that the interaction of Tf with Fe(III) is similar to that of Pu(IV), Th(IV) and Hf(IV). ([77] Duffield, 1987) They suggested that the metabolic differences observed in the case of Pu(IV) are more likely to be related to a conformational modification of the protein in the presence of exogenous metals. Harris showed
that Th(IV) binds Tf as a di-Th(IV)-Tf complex, involving three tyrosine groups, two in the C lobe and one in the N lobe. But the complexing site of the N lobe appears slightly smaller than the site located within the C lobe. This suggests that Th(IV) has steric limitation for the N site while Pu(IV) could be easily inserted into both sites (the ionic radius of Pu(IV) is \(~0.07\ \text{Å}\) smaller than Th (IV)). {[78] Harris, 1981} Combining X-Ray Absorption spectroscopy and spectrophotometry, C. Den Auwer and collaborators obtained preliminary results on the possible Tf uptake sites when the protein is exposed to U(VI), Np(IV) and Pu(IV). {[79] Den Auwer, 2005} In a comparative study between the interaction of An(IV) (Pu, Np and Th) with Tf by X-ray Absorption Spectroscopy (XAS), Jeanson et al. found some similarities between the behavior of Fe(III) and that of Np(IV), Pu(IV). {[80] Jeanson, 2010} In addition, they suggested that the complexation of actinide(IV) by apo-Tf is quantitative at physiological pH for Np and Pu, while at pH < 7 complexation is impossible. These results are in agreement with the "cycle" of Tf in vivo which complexes Fe(III) at pH = 7.4, before release it in cells at pH \(~5\). For Np and Pu, complexation may occur via a tyrosine group of the protein with the possible formation of a mixed hydroxo complex. The synergistic anion NTA (trinitriloacetate) used in the experiment to protect actinides against hydrolysis might also be involved in the metal coordination sphere as a synergistic anion, as suggested by Llorens et al. {[81] Llorens, 2005} The authors confirmed a common complexation site for Pu(IV), Np(IV) and Fe(III) while Th(IV) does not seem to interact with the protein under the experimental conditions studied. From a thermodynamic point of view, the complexation constants of An(IV) with Tf were initially assessed on the basis of UV spectroscopic measurements. Yule et al. give the complexation constant of Pu(IV)-Tf: \(\log K_{\text{Pu(IV)-Tf}} = 21.75 \pm 0.75\). {[82] Yule, 1991} Very recently, Sauge-Merle and collaborators revisited the affinity constants of Pu(IV) and Fe(III) for Tf with an original approach using CE-ICP-MS. {[83] Sauge-Merle, 2017a} Using the NTA anion as a synergistic anion and as a competitor of Tf and working at concentrations low enough to be outside the hydrolysis zone of Pu(IV), they showed that under physiological conditions, the affinity constant of the protein
C lobe (higher affinity site) for Pu(IV) is $10^4$ times higher than for Fe(III) ($\log K_{C\text{ Pu(IV)-Tf}} = 22.5 \pm 0.19$ and $\log K_{C\text{ Fe(III)-Tf}} = 18.06$, respectively). Based on the same approach, Brulfert determined very recently the complexation constant of Th(IV)-Tf, $\log K_{C\text{ Th(IV)-Tf}} = 18.65 \pm 0.19$ at pH=7, comparable to that of Fe(III) ($\log K_{1\text{ Fe(III)-Tf}} = 18.06$). ([84] Brulfert, 2018)

As already mentioned in the introduction, lanthanides (Ln(III)) have often been used as stable analogues of An(III). For example, it has been suggested that only one Gd(III) atom is complexed by Tf via the C lobe. ([85] Frausto da Silva, 1991) Various studies involving Nd(III), Pm(III), Sm(III), Eu(III), Y(III), Ho(III) and Tb(III) ions lead to the same conclusion. ([86] Luk, 1971; [87] Harris, 1986; [88] Unalkat, 1992) It is therefore reasonable to consider the same type of interaction with An(III). Like Jeanson ([80] Jeanson, 2010) with An(IV), Bauer and her co-authors explored in a work combining TRLS and XAS, the coordination modes of Cm(III) and Am(III) with Tf under variable pH conditions. ([89] Bauer, 2014) They showed that for pH > 7.4, 30% of Cm(III) binds Tf at the same complexing site as Fe(III) (in the C lobe), while at lower pH, a partially coordinated Cm(III) transferrin species is present (possibly at a nonspecific site). At physiological pH, the complexing site of Cm(III) (in agreement with that of Am(III)) is composed of two tyrosine groups (Tyr-188 and Tyr-95), a histidine group (His-249) and an aspartate group (Asp-63) as shown in Figure 4. Three carbonate, bicarbonate or hydroxide ligands may complete the coordination sphere as well as two water molecules, thus increasing the coordination number of An(III) to 9. ([90] Bauer, 2015a; [91] Bauer, 2015b)

Figure 4. Proposed structure of the Cm(III) transferrin species; X represents additional ligands, such as carbonate, bicarbonate and/or hydroxide (coordinating water molecules are not shown). ([90,91] Bauer, 2015)
Based on the determination of the complexation constants of Nd(III) and Sm(III), Harris proposed the affinity constants of Am(III) and Cm(III) \((\log K_{\text{Am(III)-Tf}} = 6.3 \pm 0.7 \text{ and } \log K_{\text{Cm(III)-Tf}} = 6.5 \pm 0.8 \text{ respectively})\) ([87] Harris, 1986) But those values are not described as site specific. They are significantly lower than for An(IV), in agreement with the preliminary studies conducted by Duffield indicating a relatively moderate affinity of An(III) for Tf. This might be explained by the large ionic radius of these cations compared to that of Fe(III) or Pu(IV). More recently, Sturzbecher-Hoene confirmed this tendency with Cm(III). He proposed two complexation constants corresponding to the two complexation sites, \(\log K_{\text{Cm(III)-Tf}} = 8.8\) and \(\log K_{\text{N Cm(III)-Tf}} = 7.0\), respectively within the C and N lobes of the protein. ([92] Sturzbecher-Hoene, 2013) He also confirmed by TRLS the involvement of the tyrosine group in the metal coordination sphere, leading to an exaltation of the luminescence of Cm(III) (an effect well known for lanthanides as the antenna effect). Finally, the affinity of the Cm(III)-Tf complex for the Tf receptor (Tf-R) may explain the rapid elimination of An(III) from the blood and its high retention rate by the liver. ([92] Sturzbecher-Hoene, 2013) Another recent study using high-performance liquid chromatography allowed the determination of the complexation constants of different Ln(III), An(IV, VI), as well as Fe(III) with the Tf-R, the Tf receptor. ([93] Deblonde, 2013) The authors confirmed the highest affinity for the receptor with Fe(III), while the Pu(IV)\textsuperscript{2-}Tf complex has the lowest affinity to the receptor (see table 1). Th(IV) and U(VI) are very well "recognized" by the receptor, while Ln(III), taken here as analogs of An(III), have an intermediate affinity.

Although Tf does not appear to be the major target protein for U(VI) in plasma, some studies have described the interaction between U(VI) and Tf. Back in the late 90's, Scapolan characterized the U(VI)-Tf complex by TRLS. ([94] Scapolan, 1998) For low concentrations of U(VI) ([U] =10\textsuperscript{-8} M), he showed the formation of a 2:1 \((U : Tf)\) complex with a global complexation constant of \(\log K_{\text{U(VI)-Tf}} = 16\). More recently, Vidaud et al. ([95] Vidaud, 2007) by combining Fourier Transform Infrared (FTIR) and UV-visible (UV-vis) spectroscopies,
showed that two tyrosine groups are involved in the oxocation coordination plane, while histidines do not seem to participate. This unsuitability of the Fe(III) ligands to bind U(VI) may be explained by the particular size and shape of the oxocation. Therefore, the lobes’ closure cannot be complete and the U(VI)-Tf complex cannot be recognized by the transferrin receptor, inhibiting/limiting thus its internalization into the cells. Montavon combined measurements in UV-vis and TRLS spectroscopies and highlighted the formation of ternary complexes with carbonate ions acting as synergistic anions. ([96] Montavon, 2009) In these conditions, the complexation constants were also assessed: log $K_C U(VI)$-Tf = 13 (high affinity site) and log $K_N U(VI)$-Tf = 12 (low affinity site). During this same time, the complexing constants of U(VI) with the proteins Tf, HSA, F and metallothionein (MT) have been reassessed on the basis of TRLS measurements. ([97] Michon, 2010) The complexation constants for U(VI)-Apo-Tf appear to be slightly higher than those observed for U(VI)-HSA, U(VI)-MT and U(VI)-F (see Table 1). More recently, Wang used density functional theory to explore the mechanisms of U(VI) binding to Tf and precised the role of the carbonate ligand. ([98] Wang, 2017) Benavides-Garcia proposed an ab initio quantum mechanical computational approach to model the IR spectrum of U(VI) complex with human Tf and brought structural insights into the U(VI)-Tf binding. ([99] Benavides-Garcia, 2009)

The case of Np(V) oxocation has been relatively underexplored since it shows a tendency to reduce to Np(IV) in vivo. Racine et al. have shown that it binds relatively weakly to apo-Tf and that citrate and carbonate anions may act as competing ligands with the metal. ([100] Racine, 2003) These results are in line with the early work published by Durbin in 1998. ([101] Durbin, 1998) The geometry of the oxocation itself (as for U(VI)) is highlighted in order to explain its relatively low interaction with the protein. One can also mention that as a general rule of thumb, Np(V) has less ability for complexation than U(VI) simply because its net positive charge is decreased.
Other serum proteins: Albumin (HAS), hemoglobin

Although HSA is not the preferred target of actinides in blood plasma, its interaction with U(VI) and Th(IV) has been explored using conventional tools: UV-vis, FTIR, TRLS and circular dichroism (CD) spectroscopies. ([102] Ali, 2016) In the case of HSA, the carbonyl and the amide groups are the main groups involved in the interaction with the metal. Moreover, using the strong fluorescence of the protein (due to the presence of tryptophan residues), the authors showed that U(VI) affinity is slightly higher than that of Th(IV) at 298K. The secondary structure of HSA seems also to be affected by the presence of the actinides since there is a decrease in α helixes and an increase in β sheets indicating partial protein unfolding.

Hemoglobin, one of the most abundant proteins present in red blood cells and composed of 4 hemic groups complexing Fe(II), is not an proven target of actinides in vivo. However, its mode of interaction with U(VI) and Th(IV) has recently been investigated. ([103] Kumar, 2016) In a way quite comparable to albumin, the authors showed that both cations interact with the protein, mainly via carbonyl and amide groups, resulting in a loss of alpha helix conformation.

Calcium binding protein: calmodulin (CaM):

As already mentioned in the previous paragraph, CaM, an ubiquitous protein found in all life, is responsible for the regulation of Ca(II), with 4 Ca binding sites and a modulator of other protein activities, by modifying its conformation when interacting with the metal. CaM is an interesting example since it has been tuned for uranyl affinity. ([104] Le Clainche, 2005) The structural environment of U(VI) and Np(V) oxocations in interaction with CaM was reported by Brulfert et al. using a combination of XAS and DFT calculations. ([105] Brulfert, 2016) Coordination occurs via two carboxyl groups (with mono- and bidentate interactions) and one carbonyl group. The coordination sphere is completed by a water molecule (at pH=3) or a hydroxyl group (at pH=6). The coordination sphere of the Np(V) is similar to that of the U(VI). The study revealed the importance of the role of little explored small ligands, such as water.
molecules or hydroxo groups in the stabilization of the actinide binding site. Pardoux and his co-authors also explored the influence of phosphorylation on the ability of proteins to bind uranyl. ([61] Pardoux, 2012) They modified the sequence of the CaM complexation site I (already showing a 1000-fold higher affinity for U(VI) than for Ca(II)), by phosphorylating the threonine residue in position 9 and by including a tyrosine group in position 7. They showed that the affinity of U(VI) is increased from 25 ± 6 nM to 5 ± 1 nM at pH = 6 and to 0.25 ± 0.06 nM at pH = 7. FTIR data are in agreement with this increase because they show that the phosphate groups are directly involved in cation complexation and that the band corresponding to the antisymmetric vibration of the oxocation undergoes a bathochromic displacement of 923 to 908 cm⁻¹, characteristic of an interaction with phosphoryl groups.

Figure 5. Structural models obtained by MD simulations for the CaM1P–U complex: A) with monodentate coordination of the deprotonated phosphoryl group, B) with bidentate coordination of the deprotonated phosphoryl group, and C) with a protonated phosphoryl group. D) Structural model of the CaM–U complex. ([106] Sauge-Merle, 2017b)

Later, Sauge-Merle et al. showed that the coordination sphere is composed of a phosphoryl group linked through a monodentate interaction to U(VI) ([106] Sauge-Merle, 2017b) As it is the case for the native protein, a monodentate bound carboxyl group and a bidentate bound carboxyl group complete the first coordination sphere of the oxocation (see figure 4).

- Hyperphosphorylated proteins: vitellogenin (Vtg), phosvitin and osteopontin (OPN).

Very recently, the importance of phosphorylations in the complexation of actinides has attracted increasing interest from the scientific community. In particular, the interaction of osteopontin (OPN) and phosvitin proteins with Th(IV) and U(VI) has been explored. OPN was first identified in 2011 as a potential biomarker of worker exposure in uranium mining. ([43]
OPN is a hyperphosphorylated protein, intrinsically disordered, involved in the metabolism of mineralization. It is secreted by several organs including the bone cells and the kidneys. Its main role is related to bone remodeling. Because the skeleton represents one of the preferred target organs for all actinides, whatever their oxidation state, the properties of OPN are important for the delineation of the mechanisms underlying the chemical toxicity of actinides in bone. The secretion of OPN by the Human Kidney 2 (HK2) cells decreases following exposure to U(VI) but on another side, the suppression of the protein does not lead to an increase in the cell sensitivity to U(VI). Following this study, the interaction of OPN with U(VI) ([45] Qi, 2014), first, and then Th(IV) ([62] Creff, 2015), was described. In the case of U(VI), Safi et al. worked with a His-pSer-Asp-Glu-pSer-Asp-Glu-Val peptide which belongs to the OPN sequence. They used a combination of spectroscopic (XAS, FTIR, TRLS), thermodynamic (Isothermal Titration Calorimetry, ITC) and theoretical (DFT calculations) tools to determine the structuration of the peptide around the U(VI). ([44] Safi, 2013) The coordination sphere of U(VI) in peptides includes a bidentate carboxylate group and a phosphate group bound in a monodentate mode. Two water molecules may complete the coordination sphere, bringing the number of coordination to 5 in the equatorial plane (see Figure 6). The similarity of the EXAFS spectra of OPN and this peptide is important and proves that a simplified peptide model could be used to mimic the protein binding site.

Figure 6. Theoretical model based on DFT calculation, for the possible U(VI) interaction site on the peptide mimicking OPN protein possible binding site. ([44] Safi, 2013)

In the case of Th(IV), because the typical coordination polyhedron of U(VI) and Th(IV) are radically different, the coordination sphere involves two monodentate phosphate groups and one bidentate carboxylate function. Four water molecules complete the Th(IV) coordination sphere, bringing the number of coordination to 8. ([62] Creff, 2015) In both cases, the
complexation site of the actinide, therefore, seems to be different from that of Ca(II), which only involves carboxylate groups. Moreover, this work confirmed the high affinity of An(IV) for phosphonate groups, perhaps explaining the very high affinity of the latter for the bone matrix itself (retention rates of 70%, 50% and 10-15%, respectively for Th(IV), Pu(IV) and U(VI)). Qi and collaborators confirmed also the importance of phosphorylation in U(VI) complexation and suggested that the protein forms stable complexes with 6 to 9 U(VI) cations. ([45] Qi, 2014) They also highlighted by CD a partial folding of the disordered protein when interacting with U(VI).

Some research groups have thus developed the design of phosphorylated peptides in order to rationalize the role of phosphorylated amino acids in their interaction with actinides, and more particularly U(VI). ([107] Starck, 2015; [108] Starck, 2017)

Quite recently, Bourrachot et al. highlighted a significant decrease in the production of vitellogenin (Vtg) in zebrafish (Danio rerio) upon U(VI) exposition. ([109] Bourrachot, 2014) Vtg is one of the most phosphorylated proteins of the living cells. At the same time, Coppin et al. defined, by TRLS, the affinity constant of the Vtg protein with U(VI) within the two protein sites: see Table 1). ([110] Coppin, 2015) He highlighted the strong affinity of U(VI) for Vtg in comparison to the other proteins: Vtg > Apo-Tf > Apo-F > HSA > MT, thus confirming the essential role of the phosphate groups. In a vibrational spectroscopy study, Li and her co-authors have already shown the increasing importance of phosphate groups in the U(VI) coordination sphere of phosvitin, a fragment of Vtg, for increasing P/U ratios. ([111] Li, 2010)

**Table 1.** Compilation of apparent affinity constants and complexation constants for various cations and proteins. Values are reported as published without any further correction.

---

III. Impact of actinides on protein conformation and function:
Metals in metalloproteins are known for their role in electron exchange, catalysis, or coordination, but they may also modulate the protein structure (locally), or protein folding. When exogenous metals are involved, the question on how these metals will affect folding and conformation (also in a dynamic way) has become a central topic.

In this field, Tf has time and again been the most explored protein by far. In 1993, using Small Angle X-ray scattering Spectroscopy (SAXS), Grosseman's investigated the conformation modification of Tf in the presence of exogenous Hf(IV) used as a non-radioactive analog of Pu(IV). He suggested that Hf(IV), and by extension all An(IV) cations (including Pu(IV)), are unable to induce closure of the C and N lobes of Tf, unlike Fe(III). ([112] Grosseman; 1992, [113] Grosseman, 1993) This first approach to the conformational modification of Tf in the presence of an exogenous metal converges with Planas-Bohne's work which highlights the inability of cells to internalize the Pu(IV)-Tf complex via the Tf-R receptor. ([114] Planas-Bohne, 1990) But more recently, Jensen demonstrated the cellular (adrenal gland cells, PC12) internalization of a Pu(IV)-Tf complex ([115] Jensen, 2011) by combining SAXS and X-ray fluorescence microscopy. In his work, Jensen showed that only the mixed PuCFeNTf complex adopts a conformation that can be recognized by the Tf-R receptor (see Figure 7). If Tf does not seem to completely block the cellular internalization of Pu, as suggested by Grosseman's early work, it nevertheless restricts access to it.

Figure 7. Metal-dependent conformations of transferrin. (a) Reconstruction of Fe$_2$Tf bound to the Tf-R (not shown). The N-terminal lobe is shown in green, and the C-terminal lobe is rendered in blue. The two Fe(III) atoms are shown in red. Adapted from PDB entry code 1SUV5. (b) Conformation of apo-Tf and the mixed PuCFeNTf complex, reconstructed from small-angle X-ray scattering. Of all the metallated Tf complexes tested, only the PuCFeNTf form binds strongly to the TfR in vitro and facilitates cellular Pu accumulation in PC12 cells via TfR-mediated endocytosis. ([115] Jensen, 2011)
More recently, Brulfert and co-authors showed, using the original method of CE-ICP-MS, that the protein conformation is not modified in the presence of An(IV) (Pu(IV) and Th(IV)). On the contrary, Fe(III) and In(III) (Fe$_2$Tf and In$_2$Tf) cations induce a closure of the protein lobes and thus a significant modification of the protein structure. ([84] Brulfert, 2018)

In her work on the interaction of U(VI) with apo-Tf, Vidaud et al. combined CD and ITC to highlight the conformational modification of the protein in the presence of uranyl. ([93] Vidaud, 2007) As presented above, the protein adopts a semi-open conformation of the N lobe (unlike Fe(III)) which excludes the interaction of the complex with the Tf-R receptor and prevents its internalization into the cell. Again, this comparison underlines the fundamental differences between the actinyl binding mode and the An(IV) binding mode.

Despite the fundamental importance of folding and, more generally, of the tertiary structure modification upon actinide complexation, experimental and computational works are still very rare. In the future, such studies based on multi-scale approaches will be essential in order to assess the function of the metalloproteins.

**Conclusion.**

Although early studies describing the retention as well as the excretion rates of actinides go back as early as the second half of the XX$^{th}$ century, little is known about their biochemical pathways. Although researchers have been interested in the identification of the actinide target proteins as early as the 40’s, in concomitance with the first biokinetics and toxicological studies, efforts in this field have been quite limited. Moreover, among the actinides, Pu(IV) and U(VI) represent the most studied elements because of their particular place in the history of the nuclear industry. However, to date, because of the increase in mining activities associated with lanthanide elements, there is growing concern about thorium exposition, as thorium is co-extracted as a by-product of lanthanide mining. ([116] Lu, 2016)
While the identification of major actinide target proteins represents an essential step towards a better understanding of their accumulation, storage and, more generally, their toxicity, through extensive mechanistic approaches are still lacking.

Affinity (a thermodynamic approach) and structure of the possible binding sites (a spectroscopic approach) is the information needed in order to interpret the biokinetic data. In this sense, transferrin, the main protein targeted by actinides in the serum, has been relatively well documented in the literature. Some studies have also focused on albumin, although it is known to have a lower affinity for actinides. Some other proteins, known for their important role in the function of major target organs, have also been explored (OPN and FETA, in bone and serum for example). Their role in the homeostasis of abundant elements in biology (like Ca or Fe for instance) has also garnered attention. More recently, hyperphosphorylated proteins (OPN, Vtg, phosvitin) have also been gaining notice from the scientific community, particularly because of the affinity of the phosphate groups for actinides but also because of the importance of phosphorylation in all biological systems.

On another note, the impact of actinides on the modulation of metalloprotein conformation and function remains relatively unexplored to date. This is surprising if one considers that the description of those modifications is essential for better understanding the biochemical mechanisms associated with the chemical toxicology of actinides.

There is still, therefore, a wide-open field for investigation on the interaction of proteins with actinides.

Acknowledgements

The authors would like to acknowledge the CEA Nuclear Toxicology program for their constant support and funding over the past 10 years. This work was also supported by the Agence Nationale pour la Recherche under the TURBO project (ANR-16-CE34-0003-01).
References


[95] Vidaud, C. Gourion-Arsiquaud, S., Rollin-Genetet, F., Torne-Celer, C., Plantevin, S., Pible, O., Berthomieu, C., Quéméneur, E.: Structural Consequences of Binding of UO22+ to
Apotransferrin: Can This Protein Account for Entry of Uranium into Human Cells?. Biochemistry 46, 2215 (2007).


As previously reported, ytterbium (Yb) resulting M size of the metals, as well as on the conformation of the Tf complex formed with Fe. Unfortunately, no comparable data is available for An(III) because of their high specificity. In contrast, a variety of spectroscopic studies have been described elsewhere. These results imply that the conformation of the TfR-mediated uptake of metal-bound Tf (Mred) and Tf in 3+ carbonate anion participating in the coordination of Fe ions, the complex FeTf, and FeTf, and the kinetic rates of intracellular Fe release from TfR-mediated intracellular iron uptake. (a) Once bound to Tf, FeTf is recognized by the TfR membrane receptor and other metal ions and is still considered a highly selective system based on the oxidation state, charge, and size of the metals, as well as on the conformation of the Tf complex formed with Fe. This is the proposed pathway for intracellular uptake. (b) Metal binding site of the Tf structure of the (Tf)N2677ff. (c) Metal binding site of the Tf horiz. analogs and organ uptake rates observed with various actinides. These results also raise the question of whether discriminating capabilities in biological systems such as the Tf/TfR metal-uptake pathway could be revisited and used to develop effective in discriminating radiotracers for An(III) and Pu isotopes must be handled in fac. Measurements were performed at 25°C. Quantum yields were determined by the optical dilute method, using an excitation wavelength of 275 nm and tyrosine as a reference. Solution Thermodynamics of Metal-Tf Solutions. Preparations of Protein Solutions. Recombinant His-tagged sTfR was produced and purified from a BHK cell expression system, as previously described. Photophysical Characterization. Procedures for the data treatment of luminescence spectra using the absorption double beam absorption spectrometer, using quartz cells of 1.00 cm path length. Emission spectra and lifetimes were acquired on a HORIBA Jobin Yvon IBH FluoroLog-3 spectrofluorimeter, as previously published methods.
Computational screening for potential uranyl-binding sites.

**Results**

Using URANTEIN we searched the PDB for pockets that could accommodate uranyl (Fig. 2). A total of 12,173 protein structures in the PDB were considered as scaffolds, on which uranyl-binding sites were searched using the oxygen library and hydrogen library. An algorithm called URANTEIN was used to complete this work efficiently (see Supplementary Information). We designed an algorithm to identify native uranyl-binding proteins and the corresponding binding sites in silico, and selectivity (see Supplementary Information) used in our program helps to establish potential hydrogen-bonding interactions between uranyl oxo and residues from the binding protein. Structures of uranyl complexes with inorganic or organic ligands were employed as scaffolds. Then, one scaffold in the library was picked for further screening (for example, the green one shown here). In step 3, the oxygen library of possible mutations and the hydrogen library of native protein were built (Supplementary Fig. 3). In step 4, for each scaffold, uranyl-binding geometries, either natively or through mutation of potential ligand residues to aspartate/asparagine or glutamate/glutamine, were employed as scaffolds. Then, one scaffold in the library was picked for further screening (for example, the green one shown here). In step 3, the oxygen

**Figure 2.**

The main steps in computational screening and design of uranyl-binding proteins.

**Step 1**

- Coordination features

**Step 2**

- Pick a scaffold

**Step 3**

- Build oxygen library and hydrogen library

**Step 4**

- Search for uranyl-binding sites

**Step 5**

- Go back to step 2 if there is no hit

- Scoring and sorting
be further divided into N1- (1–92 and 247–330), N2- (93–246), termed N-lobe (residues 1–330) and C-lobe (340–679) and can Fe by the molecular replacement method (Table 1). Similar to other SCIENTIFIC Overall structures of Fe Results closed'' conformation for holo-TF such as the resolved structure of TF such as the resolved crystal structure of apo-hTF or a ''fully-

** Figure 3. **

**Figure 1**

Iron bound transferrin families. However, the N-lobe in both
to the fold that is characteristic of the members in the transferrin
C1- (340–425 and 573–679) and C2-subdomains (426–572),
peptide linker in purple. Fe(III) ions are represented as sphere models
with subdomains N1 in blue, N2 in green, C1 in yellow, C2 in red, and

**Figure 2**

unambiguously demonstrating that Bi(III) is only located in the
density only in the N-lobe of hTF (Figure 1d and Figure S3),

**Table 1**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Fe/CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>apo-hTF</td>
<td>5265</td>
</tr>
<tr>
<td>hTF</td>
<td>5266</td>
</tr>
</tbody>
</table>

**Figure 3.**
Figure 4.

The diagram illustrates the coordination environment of a 
complex with 
metal ion (Cm$^{3+}$) bound to transferrin. The complex is
coordinated by four amino acids (two tyrosine, one his-
itive, one aspartate) and two synergistic anions.

- Tyr-188
- His-249
- Tyr-95
- Asp-63
Figure 5.
Figure 6.

Theoretical models calculated by using DFT calculations for the three possible UVI interactions with the phosphopeptide chain. One can clearly see the nonsymmetric nature of the interaction sites (a–c), and model d, which represents amino acid (A.A.) coordination to the UVI phosphorus atom.

Model c was adopted as a model to fit all the EXAFS spectra because it was the most energetically stable, whereas the distances agreed with relevant bond parameters (Table 3). The distance value for the phosphorus-related multiple-scattering paths were also necessary to fit the experimental data. The degenerate four-legged multiple-scattering path of the UVI interaction sites was finally added.

In the fitting procedure, for the U-O bond angle is not linear (eq1 and eq2). Atom O/C0 was fixed during the fit procedure. [d] Deviation from theoretical bond parameters (Table 3). The distance value for the phosphorus atom and the used fit constraints and parameters.

Table 3. Description of the paths included from DFT calculated models and the used fit constraints and parameters.

<table>
<thead>
<tr>
<th>Path Description</th>
<th>Atom Types</th>
<th>Fit Constraints</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single path of O/C0</td>
<td>bi</td>
<td>deg</td>
<td>ax</td>
</tr>
<tr>
<td>Two tightly bound O/C0</td>
<td>ax2</td>
<td>deg</td>
<td>ax1</td>
</tr>
<tr>
<td>Triple and quadruple multiple-scattering</td>
<td>P</td>
<td>eq1</td>
<td>eq2</td>
</tr>
</tbody>
</table>

Characterization of the Binding of Uranium to a Phosphorylated Peptide

Phosphorylation of 2.94

This inference is further supported by the U VI boxylate. This hypothesis is further verified when comparing the U VI interaction sites lying in the length of the U VI structure (d), a phosphoserine amino acid complex, which is more stable by 0.50 and 0.80 eV, respectively. The structural characteristics of the latter two were equivalent, the only difference being that both are in the same interaction site.
Pu inhalation may be, the risk from the general population isotope of Pu found in a patient pool in the to the generally limited body-load of any cytotoxicity. There are little data to indicate that the long-lived decay of Pu to the alpha and beta particles emitted in the toxicity is perceived to be due primarily to severe environmental toxin, with inhalation worldwide. Pu is widely recognized as a

in a variety of modalities and locations recycled at present; the rest remains stored per year, with only a fraction being generated species. In a fast neutron reactor, nuclear fuel rods include Pu. Other non

producing nuclear reactors; it is also such as neutron capture by U among the number of neutrons—all are on their rorome before activation to any other radionuclides in the same energy range, Pu is not found in your local supermarket; all Pu isotopes are radioactive, generated primarily in those acutely or chronically exposed.

Daniel J Kosman

A paper by Jensen, et al.

in this issue address clinical manifestations of its toxicity developing strategies to manage it to understand its metabolism in order to contribute to this understanding

Figure 7.

X-ray scattering, the authors demonstrated strongly for Pu TfR. 

demonstrating how and where the storage of this 'stealth' genotoxin occurs. 

demonstrating how and where the storage of this 'stealth' genotoxin occurs. 

demonstrating how and where the storage of this 'stealth' genotoxin occurs. 

demonstrating how and where the storage of this 'stealth' genotoxin occurs.

Metal-dependent conformations of transferrin. (a) Conformation of apo-Tf (the subscripts refer to first-row, high-valence chemistry as a lifetime risk of exposure to

Apo-Tf

PuFeN-Tf

Cell uptake
<table>
<thead>
<tr>
<th>Compartment</th>
<th>Fe(III)</th>
<th>Pu(IV)</th>
<th>U(VI)</th>
<th>Th(IV)</th>
<th>Np(IV, V)</th>
<th>Am(III), Cm(III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF</td>
<td>log $K_{FE(III)-Tf} = 18.06$ (Sauge-Merle, 2017)</td>
<td>log $K_{Pu(IV)-Tf} = 21.75 \pm 0.75$ (Yule, 1991)</td>
<td>log $K_{Pu(IV)-Tf} = 22.5 \pm 0.19$ (Sauge-Merle, 2017)</td>
<td>$K_d = 2.80 \mu M$ (Basset, 2013)</td>
<td>log $K_{Pu(IV)-Tf} = 21.75 \pm 0.75$ (Yule, 1991)</td>
<td>log $K_{Pu(IV)-Tf} = 22.5 \pm 0.19$ (Sauge-Merle, 2017)</td>
</tr>
<tr>
<td>Tfr</td>
<td>$K_{D1_{Pu(IV)-Tfr-R}} = 5 \text{ nM}$ and $K_{D2_{Pu(IV)-Tfr-R}} = 20 \text{ nM}$ (Deblonde, 2013)</td>
<td>$K_{D1_{Pu(IV)-Tfr-R}} = 0.28 \mu M$, $K_{D2_{Pu(IV)-Tfr-R}} = 1.8 \mu M$ (Deblonde, 2013)</td>
<td>$K_d$</td>
<td>$K_{D1_{Pu(IV)-Tfr-R}} = 0.28 \mu M$, $K_{D2_{Pu(IV)-Tfr-R}} = 1.8 \mu M$ (Deblonde, 2013)</td>
<td>$K_{D1_{Pu(IV)-Tfr-R}} = 5 \text{ nM}$ and $K_{D2_{Pu(IV)-Tfr-R}} = 20 \text{ nM}$ (Deblonde, 2013)</td>
<td>$K_{D1_{Pu(IV)-Tfr-R}} = 0.28 \mu M$, $K_{D2_{Pu(IV)-Tfr-R}} = 1.8 \mu M$ (Deblonde, 2013)</td>
</tr>
<tr>
<td>FETUA</td>
<td>$K_d = 30 \text{ nM}$ (Basset, 2013)</td>
<td>$K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td>$log K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td></td>
</tr>
<tr>
<td>CaM</td>
<td>$K_{D1_{U(VI)-CaM}} = 25 \pm 6 \text{ nM}$ to $K_{D2_{U(VI)-CaM}} = 5 \pm 1 \text{ nM}$ pH 6</td>
<td>$K_{D1_{U(VI)-CaM}} = 25 \pm 0.06 \text{ nM}$ pH 7 (Pardoux, 2016)</td>
<td>log $K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td></td>
</tr>
<tr>
<td>HSA</td>
<td>$log K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td>$log K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-HSA}} = 6.1$, log $K_{D2_{U(VI)-HSA}} = 4.8$ (Michon, 2010)</td>
<td></td>
</tr>
<tr>
<td>SUP</td>
<td>$K_d = 7.4 \text{ fM}$ (Zhou, 2014)</td>
<td>log $K_{D1_{U(VI)-MT}} = 6.5$, log $K_{D2_{U(VI)-MT}} = 5.6$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-MT}} = 5.6$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-MT}} = 6.5$, log $K_{D2_{U(VI)-MT}} = 5.6$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-MT}} = 6.5$, log $K_{D2_{U(VI)-MT}} = 5.6$ (Michon, 2010)</td>
<td></td>
</tr>
<tr>
<td>MT</td>
<td>$log K_{D1_{U(VI)-MT}} = 6.5$, log $K_{D2_{U(VI)-MT}} = 5.6$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-MT}} = 6.5$, log $K_{D2_{U(VI)-MT}} = 5.6$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-MT}} = 6.5$, log $K_{D2_{U(VI)-MT}} = 5.6$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-MT}} = 6.5$, log $K_{D2_{U(VI)-MT}} = 5.6$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-MT}} = 6.5$, log $K_{D2_{U(VI)-MT}} = 5.6$ (Michon, 2010)</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>log $K_{D1_{U(VI)-f}} = 5.3$, log $K_{D2_{U(VI)-f}} = 3.9$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-f}} = 5.3$, log $K_{D2_{U(VI)-f}} = 3.9$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-f}} = 5.3$, log $K_{D2_{U(VI)-f}} = 3.9$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-f}} = 5.3$, log $K_{D2_{U(VI)-f}} = 3.9$ (Michon, 2010)</td>
<td>log $K_{D1_{U(VI)-f}} = 5.3$, log $K_{D2_{U(VI)-f}} = 3.9$ (Michon, 2010)</td>
<td></td>
</tr>
<tr>
<td>Vtg</td>
<td>log $K_{D1_{U(VI)-Vtg}} = 4.9 \pm 1.1$, log $K_{D2_{U(VI)-Vtg}} = 4.9 \pm 1.1$ (Coppin, 2015)</td>
<td>log $K_{D1_{U(VI)-Vtg}} = 4.9 \pm 1.1$, log $K_{D2_{U(VI)-Vtg}} = 4.9 \pm 1.1$ (Coppin, 2015)</td>
<td>log $K_{D1_{U(VI)-Vtg}} = 4.9 \pm 1.1$, log $K_{D2_{U(VI)-Vtg}} = 4.9 \pm 1.1$ (Coppin, 2015)</td>
<td>log $K_{D1_{U(VI)-Vtg}} = 4.9 \pm 1.1$, log $K_{D2_{U(VI)-Vtg}} = 4.9 \pm 1.1$ (Coppin, 2015)</td>
<td>log $K_{D1_{U(VI)-Vtg}} = 4.9 \pm 1.1$, log $K_{D2_{U(VI)-Vtg}} = 4.9 \pm 1.1$ (Coppin, 2015)</td>
<td></td>
</tr>
</tbody>
</table>